CRISPR Gene Therapy

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Contributor: Udani Gamage , Kesari Warnakulasuriya , Sonali Hansika , Gayathri N. Silva

β-Thalassemia is an inherited hematological disorder that results from genetic changes in the β-globin gene, leading to the reduced or absent synthesis of β-globin. The emergence of the CRISPR/Cas9 genome editing platform has opened the door for precision gene editing and can serve as an effective molecular treatment for a multitude of genetic diseases. Investigational studies were carried out to treat β-thalassemia patients utilizing CRISPR-based CTX001 therapy targeting the fetal hemoglobin silencer BCL11A to restore γ-globin expression in place of deficient β-globin.

β-thalassemia β-globin γ-globin BCL11A CRISPR/Cas9 technology CTX001 therapy

1. Gene Therapy as a Promising Cure for the Acute form of β-Thalassemia

Current gene therapy approaches offer great therapeutic promise with the most remarkable clinical outcomes for inherited or acquired disorders including cancer, viral infections, and recessive genetic disorders such as thalassemia, sickle cell anemia, cystic fibrosis and hemophilia [1][2]. The goal of this technique is to correct defective genes by administering functional genetic material into cells to produce a lasting therapeutic effect. Gene therapy strategies can be divided into two main categories based on their mode of gene delivery, namely, ex vivo and in vivo gene delivery. Ex vivo gene therapy is a novel approach that entails harvesting cells from the patient, genetically modifying them in a laboratory and transplanting the tailored cells back into the target tissue. This method offers a major advantage over the in vivo gene delivery method due to the ability to fully characterize and eliminate deleterious properties of the altered cells before transplantation [3]. The transplanted genetically modified cells function in secreting and disseminating targeted proteins into the surrounding environment [4]. In contrast, the in vivo strategy delivers DNA directly to resident cells of the target tissue, typically via a viral vector [2]. Replication-deficient viral vectors (e.g., adenoviruses, adenoviral associated viruses, retroviruses, and lentiviruses) can function as gene delivery vehicles to introduce genetic material to target cells such as germline and somatic cells [5]. This technology is now being widely applied in clinical trials to treat both inherited and acquired disorders [4].

Viral vectors serve as a popular gene delivery method for β-thalassemia. For instance, lentiviruses were engineered to be excellent vector candidates for the efficient transfer of HBB in both human and animal models. In multiple mouse and primate models with β-thalassemia major [6] and intermedia, lentiviral mediated β-globin gene transfer leads to alleviation of anemia and subsequent organ damage, making recombinant lentiviruses one of the

most effective vector systems for β-thalassemia gene therapy [7][8]. Lentiviral vectors are considered a successful application for clinical trials as a result of their ability for efficient transduction of cells, such as CD34+, that undergo limited proliferation, as well as non-dividing cells with enhanced genomic stability [8][9]. In 2006, human clinical research was launched (LG001 study) utilizing lentiviral vectors for β-hemoglobinopathies [9][10]. HPV569 lentiviral vector 35 was employed to transfer the β-globin gene comprising the β-globin locus control region (LCR), which regulates the expression of the β-globin gene in autologous hematopoietic stem cells of β-thalassemic patients [10]. This therapy was clinically successful and eliminated the requirement for long-term transfusion after 1 year and was sustained for almost 8 years. The total hemoglobin level was maintained at a steady state around 8 g/dL after 2–8 years of the treatment. Furthermore, non-hematological or drug-product-related adverse events were not reported upon infusion [11].

2. Advances in CRISPR Gene Therapy Hold Great Promise as an Effective One-Time Treatment Option for TDT

CRISPR is a breakthrough RNA-guided genome editing technology of the 21st century that holds a great therapeutic promise against hereditary blood diseases [12]. CRISPR-mediated adaptive immune systems were first discovered in bacteria that provide protection against invading foreign genetic elements [13][14][15][16]. The bacterial genome contains short repetitive sequences known as CRISPR arrays, separated by non-repetitive protospacer sequences that will be transcribed and processed into matured CRISPR RNA (crRNA) sequences that are homologous to the invading genetic material. Assembly of an active CRISPR/Cas effector complex requires the incorporation of mature crRNA that guides the effector complex to the invading nucleic acids and a separately encoded multi-domain nuclease called Cas (Class I nuclease) or a set of Cas nucleases (Class II nucleases) [15]. Target recognition is achieved by the extensive complementarity between the invader DNA and crRNA sequence, leading to the Cas-dependent double-stranded cleavage of the crRNA-foreign DNA complex [13][17]. The type II CRISPR system comprising a single effector nuclease Cas9 is the simplest and best understood CRISPR system that is widely adopted in genome engineering applications [13][14][17]. The Type II system requires a second scaffold RNA molecule called trans-activating crRNA (tracrRNA) that base pairs with crRNA to form a dual guide RNA structure (gRNA), which is essential for target recognition and crRNA-guided DNA cleavage, preceding the protospacer adjacent motif (PAM) present in the target sequence [15][18][19]. The remarkable programmable capacity of type II systems was harnessed to develop powerful gene editing tools to target any desired DNA sequence by customizing the crRNA sequence. While crRNA and tracrRNA are separately encoded in nature, scientists have engineered a chimeric single-guide RNA (sgRNA) molecule by fusing crRNA with tracrRNA. In CRISPR-based therapeutic approaches, safe and efficient delivery of the CRISPR/Cas9 system into target cells is an important consideration. CRISPR/Cas9 system can be introduced into isolated target cells from β-thalassemia patients through viral or non-viral delivery vectors where the RNA-dependent DNA cleavage is carried out by Cas9 after recognition of the target sequence by sgRNA. Electroporation and microinjection can be indicated as safe non-vector-based methods for CRISPR delivery [1][20][21].

3. CTX001 Therapy

CTX001 is an investigational genetically modified cell therapy studied by CRISPR therapeutics (Cambridge, MA, USA) and Vertex Pharmaceuticals (Boston, MA, USA) for inherited hematological disorders such as sickle cell disease (SCD) and TDT. Currently, CTX001 clinical trials are recruiting patients from the United States. It is a non-viral based, ex vivo CRISPR/Cas9-mediated site-specific editing in the erythroid-specific enhancer binding site of BCL11A in autologous CD34+ hematopoietic stem and progenitor cells (HSPC) to increase γ-globin gene expression and, subsequently, increase the HbF level in patients. The CTX001 infusion was shown to yield 80% allele modification in the BCL11A locus without evidence of off-target editing [22]. The upregulation of HbF reduced the transfusion requirement and anemic condition in β-thalassemia and lessened clinical complications in SCD patients, such as vaso-occlusive crises (VOCs) [23].

CTX001 therapy has led to the disruption of the erythroid-specific enhancer binding site of BCL11A, resulting in the downregulation of BCL11A expression. As a result, transcriptional silencing of γ-globin by BCL11A is reversed, and the γ-globin expression is reactivated to increase HbF and total Hb levels in CTX001 received TDT patients. Maintenance of elevated levels of HbF and total Hb levels were also observed in patients over time [24][25]. During the 21.5 months after receiving CTX001 therapy, the very first patient showed 32 adverse events, but they were considered to be in the early stages of severity. Some patients in early clinical trials showed severe adverse events such as haemophagocytic lymphohistiocytosis and acute respiratory distress syndrome [22][23]. Overall, these adverse events were found to be correlated with busulfan myeloablation and autologous HSPC transplantation [26]. Other detrimental effects observed included pneumonia and sepsis with neutropenia, veno-occlusive disease and sinusoidal obstruction syndrome, abdominal discomfort, and cholelithiasis, as well as non-serious consequences of lymphopenia [22]. The occurrence of these effects could be attributed to the delay in the recuperation of lymphocytes due to CD4+ T cell enrichment after CTX001 therapy [22][27]. The CLIMB-Thal-111 study was formulated to appraise the safety and efficacy of CTX001 dosing for patients of 12–35 years of age while endorsing long term-follow up protocols of up to 15 years (CLIMB-Thal-131) [28].

In addition to CTX001 trials, ST-400 is another type of investigational ex vivo autologous cell therapy that utilizes gene editing to increase the production of HbF. It is a phase 1/2 clinical study that relies on Sangamo's Zinc Finger Nuclease (ZFN) technology, which facilitates targeted genome editing. ST-400 trials follow a similar strategy as CTX001 to disrupt the enhancer of the *BCL11A* gene, subsequently reactivating the γ-globin gene expression [29]. Nevertheless, ongoing CTX001 trials with positive outcomes suggest that this novel therapy has considerable potential to be used in treating TDT patients [6]. However, adverse events such as unintended off-target editing, inefficient gene delivery, and autoimmune reactions must receive considerable attention during clinical use. Hence, careful monitoring and follow-up of clinical efficacy, genotoxicity, and other safety protocols are imperative. Comprehensive pre-clinical studies and laboratory and computational-based techniques must be employed to identify the risk of observing possible adverse events [22]. Further progress in experimentation and understanding of the underlying molecular concepts about the safety and efficacy of CTX001 therapy will shed light on therapy, improving the quality of life of patients.

4. Relative Merits

CRISPR-mediated site-specific gene editing technology has brought about a transformative and promising treatment method for TDT. This approach avoids the time-consuming steps of protein engineering, such as altering and generating site-specific nucleases that recognize a specific DNA sequence [30], while enhancing the target design simplicity and reducing implementation time. Therefore, CRISPR/Cas9 has proven to be a simple, efficient, and cost-effective gene therapy technique for inherited blood disorders [31]. Unlike viral-vector-mediated gene insertion, genome editing by CRISPR does not require the use of integrating vectors, since transitory production of a specific endonuclease is able to induce the required DNA cleavage.

Even though CRISPR technology alleviates some of the hindrances associated with traditional gene therapy, it has some drawbacks in the clinical setting. A major concern of this method is the higher frequency of unintended genome cleavages, resulting in off-target mutagenesis rendering unforeseen and undesirable outcomes during therapeutic trials. Off-target effects impose life-threatening risks due to deleterious genetic changes and deprivation of gene function, eventually leading to unfavorable phenotypes. At present, researchers are in the process of exploring different strategies to mitigate this issue [21][32]. Off-target effects were assessed using a variety of methodologies during CRISPR-gene therapy for β-thalassemia [33]. Several studies have shown that the use of high-fidelity enzymes and a double-nicking strategy can significantly decrease off-target activity while preserving acceptable efficiency for editing the target genome [33][34]. For instance, recently, an efficient Cas9 double nickase mediated gene targeting strategy was employed for iPSC of a patient with β-thalassemia mutation (IVSII-1 G > A) by integrating catalytic mutant Cas9D10A nickase with a pair of sgRNAs complementary to the target sequence [34][35]. The optimization of single guide RNAs can also improve the efficacy and accuracy of gene editing [32][36]. The application of in silico methods for predicting and quantifying off-targets is important in overcoming this problem [32][36][37].

However, appealing uses of this approach raise certain challenges on ethical, social, and safety protocols, specifically when utilizing it in the clinical platform [38]. The uncertainty of whether this therapy causes a permanent genetic alteration in the organism and whether the modified gene passes to subsequent generations is a major concern. Even if the genome is altered as planned and the intended functional problem is resolved at the specified time, it is uncertain whether the complicated interaction between genetic information and biological phenotypes would be clearly defined. Undesired clinical outcomes may arise due to uncertainty associated with genome modifications in complex biological systems creating dilemmas in ethical deliberations [38][39]. Therefore, comprehensive discussions on the societal ramifications involving scientists, legislators, and ethicists are required as the utilization and patenting of gene therapy techniques for treatment purposes has caused a rift within scientific communities [38]. However, it is noteworthy that each of these ongoing and prospective advancements in designing and enhancing CRISPR/Cas9-based systems for genome editing will undoubtedly revolutionize the field of medicine in the upcoming decade and will possibly provide a permanent cure for β-hemoglobinopathies.

References

1. Gonçalves, G.A.R.; Paiva, R.D.M.A. Gene Therapy: Advances, Challenges and Perspectives. Einstein 2017, 15, 369–375.
2. Selkirk, S.M. Gene Therapy in Clinical Medicine. Postgrad. Med. J. 2004, 80, 560–570.
3. Canver, M.C. Evaluation of the Clinical Success of Ex Vivo and In Vivo Gene Therapy—Journal of Young Investigators. Available online: https://www.jyi.org/2009-january/2017/10/2/evaluation-of-the-clinical-success-of-ex-vivo-and-in-vivo-gene-therapy (accessed on 9 April 2022).
4. Soofiyani, S.R.; Baradaran, B.; Lotfipour, F.; Kazemi, T.; Mohammadnejad, L. Gene Therapy, Early Promises, Subsequent Problems, and Recent Breakthroughs. Adv. Pharm. Bull. 2013, 3, 249–255.
5. Lundstrom, K. Viral Vectors in Gene Therapy. Diseases 2018, 6, 139–143.
6. Karamperis, K.; Tsoumpeli, M.T.; Kounelis, F.; Koromina, M.; Mitropoulou, C.; Moutinho, C.; Patrinos, G.P. Genome-Based Therapeutic Interventions for β-Type Hemoglobinopathies. Hum. Genom. 2021, 15, 32.
7. Nienhuis, A.W.; Persons, D.A. Development of Gene Therapy for Thalassemia. Cold Spring Harb. Perspect. Med. 2012, 2, a011833.
8. Li, W.; Xie, S.; Guo, X.; Gong, X.; Wang, S.; Lin, D.; Zhang, J.; Ren, Z.; Huang, S.; Zeng, F.; et al. A Novel Transgenic Mouse Model Produced from Lentiviral Germline Integration for the Study of β-Thalassemia Gene Therapy. Haematologica 2008, 93, 356–362.
9. Milone, M.C.; O'Doherty, U. Clinical Use of Lentiviral Vectors. Leukemia 2018, 32, 1529–1541.
10. Bank, A.; Dorazio, R.; Leboulch, P. A Phase I/II Clinical Trial of β-Globin Gene Therapy for β-Thalassemia. Ann. N. Y. Acad. Sci. 2005, 1054, 308–316.
11. Negre, O.; Eggimann, A.V.; Beuzard, Y.; Ribeil, J.A.; Bourget, P.; Borwornpinyo, S.; Hongeng, S.; Hacein-Bey, S.; Cavazzana, M.; Leboulch, P.; et al. Gene Therapy of the β-Hemoglobinopathies by Lentiviral Transfer of the BA(T87Q)-Globin Gene. Hum. Gene Ther. 2016, 27, 148.
12. Amjad, F.; Fatima, T.; Fayyaz, T.; Khan, M.A.; Qadeer, M.I. Novel Genetic Therapeutic Approaches for Modulating the Severity of β-Thalassemia (Review). Biomed. Rep. 2020, 13, 48.
13. Xiao-Jie, L.; Hui-Ying, X.; Zun-Ping, K.; Jin-Lian, C.; Li-Juan, J. CRISPR-Cas9: A New and Promising Player in Gene Therapy. J. Med. Genet. 2015, 52, 289–296.
14. Kim, E.J.; Kang, K.H.; Ju, J.H. CRISPR-Cas9: A Promising Tool for Gene Editing on Induced Pluripotent Stem Cells. Korean J. Intern. Med. 2017, 32, 42.
15. Newsom, S.; Parameshwaran, H.P.; Martin, L.; Rajan, R. The CRISPR-Cas Mechanism for Adaptive Immunity and Alternate Bacterial Functions Fuels Diverse Biotechnologies. Front. Cell. Infect. Microbiol. 2021, 10, 898.

16. Mojica, F.J.M.; Rodriguez-Valera, F. The discovery of CRISPR in archaea and bacteria. FEBS J. 2016, 283, 3162–3169.
17. Lino, C.A.; Harper, J.C.; Carney, J.P.; Timlin, J.A. Delivering CRISPR: A Review of the Challenges and Approaches. Drug Deliv. 2018, 25, 1234–1257.
18. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science 2012, 337, 816–821.
19. Gasiunas, G.; Barrangou, R.; Horvath, P.; Siksnys, V. Cas9-CrRNA Ribonucleoprotein Complex Mediates Specific DNA Cleavage for Adaptive Immunity in Bacteria. Proc. Natl. Acad. Sci. USA 2012, 109, E2579–E2586.
20. Khalil, A.M. The Genome Editing Revolution: Review. J. Genet. Eng. Biotechnol. 2020, 18, 68.
21. Uddin, F.; Rudin, C.M.; Sen, T. CRISPR Gene Therapy: Applications, Limitations, and Implications for the Future. Front. Oncol. 2020, 10, 1387.
22. Frangoul, H.; Altshuler, D.; Cappellini, M.D.; Chen, Y.-S.; Domm, J.; Eustace, B.K.; Foell, J.; de la Fuente, J.; Grupp, S.; Handgretinger, R.; et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. N. Engl. J. Med. 2021, 384, 252–260.
23. Frangoul, H.; Bobruff, Y.; Cappellini, M.D.; Corbacioglu, S.; Fernandez, C.M.; De La Fuente, J.; Grupp, S.; Handgretinger, R.; Ho, T.W.; Imren, S.; et al. Safety and Efficacy of CTX001TM in Patients With Transfusion-Dependent β-Thalassemia or Sickle Cell Disease: Early Results From the CLIMB THAL-111 and CLIMB SCD-121 Studies of Autologous CRISPR-CAS9-Modified CD34 + Hematopoietic Stem and Progenitor Cells. In Proceedings of the 62nd Annual American Society of Hematology Meeting, Washington, DC, USA, 6 December 2020.
24. CTX001 for Treatment of Sickle Cell Disease and Other Blood Disorders. Available online: https://sicklecellanemianews.com/ctx001-sickle-cell-disease (accessed on 9 April 2022).
25. Humbert, O.; Samuelson, C.; Kiem, H.P. CRISPR/Cas9 for the Treatment of Haematological Diseases: A Journey from Bacteria to the Bedside. Br. J. Haematol. 2021, 192, 33–49.
26. Grupp, S.; Bloberger, N.; Campbell, C.; Carroll, C.; Hankins, J.S.; Ho, T.W.; Imren, S.; Lu, Y.; Mapara, M. CTX001 for sickle cell disease: Safety and efficacy results from the ongoing Climb SCD-121 study of autologous CRISPR-Cas9-modified CD34+ hematopoietic stem and progenitor cells. HemaSphere 2021, 5, 365.
27. Nachbaur, D.; Kropshofer, G.; Heitger, A.; Latzer, K.; Glassl, H.; Ludescher, C.; Nussbaumer, W.; Niederwieser, D. Phenotypic and Functional Lymphocyte Recovery After CD34+-Enriched Versus Non-T Cell-Depleted Autologous Peripheral Blood Stem Cell Transplantation. J. Hematother. Stem Cell Res. 2004, 9, 727–736.

28. Vertex and CRISPR Therapeutics Present New Data in 22 Patients with Greater Than 3 Months Follow-Up Post-Treatment with Investigational CRISPR/Cas9 Gene-Editing Therapy, CTX001TM at European Hematology Association Annual Meeting|Vertex Pharmaceuticals. Available online: https://investors.vrtx.com/news-releases/news-release-details/vertex-and-crispr-therapeutics-present-new-data-22-patients (accessed on 9 April 2022).
29. A Study to Assess the Safety, Tolerability, and Efficacy of ST-400 for Treatment of Transfusion-Dependent Beta-Thalassemia (TDT)—Full Text View—ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/NCT03432364?term=sangamo&cond=thalassemia&rank=1 (accessed on 30 January 2023).
30. Jiang, F.; Doudna, J.A. CRISPR–Cas9 Structures and Mechanisms. Annu. Rev. Biophys. 2017, 46, 505–529.
31. Zhang, D.; Hussain, A.; Manghwar, H.; Xie, K.; Xie, S.; Zhao, S.; Larkin, R.M.; Qing, P.; Jin, S.; Ding, F. Genome Editing with the CRISPR-Cas System: An Art, Ethics and Global Regulatory Perspective. Plant Biotechnol. J. 2020, 18, 1651–1669.
32. Naeem, M.; Majeed, S.; Hoque, M.Z.; Ahmad, I. Latest Developed Strategies to Minimize the Off-Target Effects in CRISPR-Cas-Mediated Genome Editing. Cells 2020, 9, 1608.
33. Frati, G.; Miccio, A. Genome Editing for β-Hemoglobinopathies: Advances and Challenges. J. Clin. Med. 2021, 10, 482.
34. Ran, F.A.; Hsu, P.D.; Lin, C.Y.; Gootenberg, J.S.; Konermann, S.; Trevino, A.E.; Scott, D.A.; Inoue, A.; Matoba, S.; Zhang, Y.; et al. Double Nicking by RNA-Guided CRISPR Cas9 for Enhanced Genome Editing Specificity. Cell 2013, 154, 1380–1389.
35. Alateeq, S.; Ovchinnikov, D.; Tracey, T.; Whitworth, D.; Al-Rubaish, A.; Al-Ali, A.; Wolvetang, E. Identification of On-Target Mutagenesis during Correction of a Beta-Thalassemia Splice Mutation in IPS Cells with Optimised CRISPR/Cas9-Double Nickase Reveals Potential Safety Concerns. APL Bioeng. 2018, 2, 046103.
36. Tycko, J.; Myer, V.E.; Hsu, P.D. Methods for Optimizing CRISPR-Cas9 Genome Editing Specificity. Mol. Cell 2016, 63, 355–370.
37. Vadolas, J.; Glaser, A.; McColl, B. The Therapeutic Potential of Genome Editing for β-Thalassemia. F1000Research 2015, 4.
38. Brokowski, C.; Adli, M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. J. Mol. Biol. 2019, 431, 88–101.
39. Shinwari, Z.K.; Tanveer, F.; Khalil, A.T. Ethical Issues Regarding CRISPR Mediated Genome Editing. Curr. Issues Mol. Biol. 2017, 26, 103–110.